

CLAIMS

1. A method for determining the ambient concentrations of a plurality of analytes in a liquid sample of volume V litres, comprising

5 loading a plurality of different binding agents, each being capable of reversibly binding an analyte which is or may be present in the liquid sample and is specific for that analyte as compared to the other components of the liquid sample, onto a support means at a plurality of

10 spaced apart locations such that each location has not more than $0.1 V/K$ moles of a single binding agent, where K litres/mole is the equilibrium constant of the binding agent for the analyte;

15 contacting the loaded support means with the liquid sample to be analysed, such that each of the spaced apart locations is contacted in the same operation with the liquid sample, the amount of liquid used in the sample being such that only an insignificant proportion of any analyte present in the liquid sample becomes bound to the

20 binding agent specific for it, and

measuring a parameter representative of the fractional occupancy by the analytes of the binding agents at the spaced apart locations by a competitive or non-competitive assay technique using a site-recognition reagent for each

25 binding agent capable of recognising either the unfilled binding sites or the filled binding sites on the binding agent, said site-recognition reagent being labelled with a marker enabling the amount of said reagent in the particular location to be measured.

30 2. A method as claimed in claim 1 wherein each of the spaced apart locations has less than $0.01 V/K$ moles of a single binding agent.

3. A method as claimed in claim 1 wherein the binding agents used have equilibrium constants for the analytes of

from 10^8 to 10^{13} litres per mole.

4. A method as claimed in claim 1 wherein the binding agents used have equilibrium constants for the analytes of the order of 10^{10} or 10^{11} litres per mole.

5 5. A method as claimed in claim 1 wherein the volume of the liquid sample is not more than 0.1 litre.

6. A method as claimed in claim 1 wherein the volume of the liquid sample is 400 to 1000 microlitres.

7. A method as claimed in claim 1 wherein the binding 10 agents loaded onto the support means are antibodies for the analytes whose concentrations are to be determined.

8. A method as claimed in claim 1 wherein the binding agents are labelled with markers enabling the concentration levels of the binding agent to be measured.

15 9. A method as claimed in claim 8 wherein the binding agents and the site-recognition reagents are labelled with fluorescent markers such that at the individual spaced apart locations the assay technique for measuring fractional occupancy of the binding agents measures the ratios of 20 the signals emitted by the fluorescent markers.

10. A device for use in determining the ambient concentrations of a plurality of analytes in a liquid sample of volume V litres, comprising a solid support means having located thereon at a plurality of spaced apart 25 locations a plurality of different binding agents, each binding agent being capable of reversibly binding an analyte which is or may be present in the liquid sample and is specific for that analyte as compared to the other components of the liquid sample, each location having not 30 more than $0.1 V/K$ moles of a single binding agent, where K litres/mole is the equilibrium constant of that binding

agent for reaction with the analyte to which it is specific.

11. A kit for use in determining the ambient
5 concentration of a plurality of analytes in a liquid sample
of volume V litres, comprising

a solid support means having located thereon at a plurality of spaced apart locations a plurality of different binding agents, each binding agent being capable
10 of reversibly binding an analyte which is or may be present in the liquid sample and is specific for that analyte as compared to the other components of the liquid sample, each location having not more than $0.1 V/K$, preferably less than $0.01 V/K$, moles of a single binding agent, where K
15 litres/mole is the equilibrium constant of that binding agent for reaction with the analyte to which it is specific,

a plurality of standard samples containing known concentrations of the analytes whose concentrations in the
20 liquid sample are to be measured, and

a set of labelled site-recognition reagents for reaction with filled or unfilled binding sites on the binding agents.

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